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(54) PRODUCTION OF PROTEIN ISOLATES

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This invention relates to a process for producing protein isolates, free from or with a low content of phytic acid, from *brassica* or *crambe* seeds. The term "phytic acid" is used herein to mean inositol hexaphosphoric acid, its salts or other compounds thereof.

Several species of *Brassica* are grown as oilseed plants in many areas of the world. The main oilseed plants grown in Sweden and Poland are rape and turnip rape (*Brassica napus* and *Brassica campestris*). Canada is the largest producer in the world of turnip rape. Also crambe (*Crambe abyssinica*) is grown in, among other countries, U.S.A., as an oilseed plant. In India and Pakistan, substantial quantities of "brown mustard" (*Brassica juneca*) are grown besides rape and turnip rape, and also smaller amounts of white and black mustard (*Brassica hirta* or *Sinapis alba* and *Brassica nigra*). The seeds of these plants contain, besides oil, a large amount of protein, which from a nutritional point of view, has a very well balanced amino acid composition. Because of their high protein content and the suitable amino acid composition of the protein, these seeds are potentially attractive as raw materials for the manufacture of protein products for use in food. Such products can be of several different types; one of the more advanced is a protein isolate.

Processes for the manufacture of protein isolates from defatted soybeans and some other oilseeds are known. In these processes, the defatted seed is usually dispersed in an aqueous alkaline solution, which solubilizes most of the protein. After separation of the undissolved residue, the protein is precipitated by the addition of acid. The precipitate obtained constitutes the protein isolate. It has a higher protein content and a lower fibre content than products prepared by a more or less selective leaching of non-protein components of the seed.

Our investigations have shown that these known procedures cannot generally be used for the preparation of protein isolates with good physical properties from seeds of *Brassica* and *Crambe* species. The production of protein isolates from these species in high yield and having the desired properties, is possible, as explained below, only if the different steps of the isolation process are conducted under certain carefully controlled conditions. This is because the relationship between the process conditions used, the yield of product, and the properties of the product is determined not only by the morphology of the seeds and the composition of the proteins therein, but also by the presence of other objectionable constituents in the seed; and seeds of *Brassica* and *Crambe* species differ in these respects from other oilseeds.

Seeds of *Brassica* and *Crambe* species contain relatively large amounts of phytic acid. The phytic acid content varies with the seed species, the content of fertilizers in the soil and the ripeness of the seeds when harvested. Phytic acid contents from 2.2 to 3.9% have been found in defatted *Brassica* seeds. For defatted soybeans phytic acid contents of 1.7%, and for peanuts contents of 1.0%, have been reported.

It is known that phytic acid forms strong complexes with proteins as well as with many other substances. These complexes are often poorly soluble over a broad pH range. Therefore, protein products with a high content of phytic acid, have properties different from those of products with a similar composition but with a low content of phytic acid.

The presence of phytic acid in protein products not only influences the properties of the product, for example its solubility and water binding capacity, but also its nutritional value. Phytic acid can effect the nutritional value of the product either by forming complexes with the protein, thus obstructing the enzymatic digestion of the protein (barre, R. *Ann. pharm. franc. 14* (1956) 182), or by reacting with calcium, magnesium, copper, zinc or iron in the food and so obstructing the adsorption of these important minerals. The human intestinal system contains no enzymes able to hydrolyze the phytic acid. At the pH values prevailing in the intestine the phytic acid forms difficultly soluble complexes with the above-mentioned metal ions. It is known that, as a result of this, consumption of food rich in phytic acid may lead to mineral deficiency. Consumption of such food increases the risk of iron deficiency, for example. Rickets can be caused by a shortage of calcium in the food and it has been established that consumption of food rich in phytic acid results in a negative calcium balance (Rheinhold, J. G., et al., *The Lancet* (1973) p. 283). Consumption of such food, therefore, can have a rachitic effect. The rachitic effect of phytic acid was observed in Ireland during the Second World War, where the frequency of rickets in children increased by 50% when they were fed wheat which had been subjected to extended grinding (Laurent, S.-O., *Finska Kemists. Medd.* (Suomen Kemistis, Tied.) *64* (1955) 12). This can be explained by the fact that the major part of the phytic acid in the wheat is located in the husk and only a minor part in the interior of the grain. In *Brassica* and *Crambe* seeds, the phytic acid is, however, mainly located in the interior parts. Detailed investigations of these seeds have shown that the main part of the phosphorus compounds are located in globoid particles, which are distributed in the protein-rich aleuron grains (von Hofsten. A., *Physiologia Plantarum 29* (1), (1973) 76). It has also been observed that a diet rich in phytic acid will lower the level of zinc in the blood plasma of humans, and increase the content in the faeces (Rheinhold et al., *The Lancet*, 1973, p. 283). It has been established that a decrease in the zinc content of the blood plasma in rats caused by, for example, insufficent zinc supply in the food, causes a very high frequency of foetus malformations (hurley, L. S. *Am. J. Clin. Nutr. 22* (1969) 1332). Therefore, in the production of protein isolates from materials with a high phytic acid content, it is important from many points of view to use process conditions which result in products practically free from phytic acid.

We have now found that by extracting the protein from defatted *Brassica* or *Crambe* seeds at a pH in the range of from 10.5 to 12, separating the insoluble material, and precipitating the dissolved proteins from the solution by addition of acid and a precipitation aid, it is possible to obtain a protein isolate which is substantially free of phytic acid in a high yield.

According to the present invention, therefore, there is provided a process for the preparation of a protein isolate substantially free from phytic acid (as herein defined), which comprises:

(a) extracting protein from defatted seeds of the genera *Brassica* or *Crambe* in an aqueous medium having a pH in the range of from 10.5 to 12 and a temperature of from 0 to 70°C,

(b) separating non-dissolved material to obtain a solution containing dissolved proteins,

(c) precipitating proteins from the solution by adding an acid and a precipitation aid,

(d) separating the precipitate obtained, and

(e) washing the precipitate to remove remaining mother liquor.

In addition to phytic acid, another objectionable constituent of *Brassica* and *Crambe* seeds is glucosinolates. These are present to the extent of 3 to 4% in the seeds of certain *Brassica* and *Crambe* species; they are water-soluble substances which, under the influence of enzymes, myrosinase, present in the seed, are hydrolysed under certain conditions to give water-soluble and water-insoluble toxic substances which exhibit, for example, goitrogenic effects. Glucosinolate hydrolysis products may react with the protein present in the seeds or may be dissolved in the oil present in the seeds. In the latter case, they may act as poisons for the catalysts used in hydrogenation of the oil.

Glucosinolates and their hydrolysis products do not, however, pose as great a problem as phytic acid since glucosinolates are not thermselves toxic and the myrosinase system is not active at the pH's used in the process according to the invention, 10.5 to 12, so that hydrolysis products are not formed and are, therefore, not present in the protein isolates obtained. Additionally, the defatting to which the seeds must be subjected before carrying out the process according to the invention is preferably carried out at an elevated temperature and the preferred temperatures for this purpose are such as to deactivate the myrosinase. A heat

treatment separate from the defatting treatment may also be used.

Heat treatment of the seeds can lead to a substantial decrease in the solubility of the seed proteins if the temperature is too high. Experiments have shown, however, that it is possible to choose heat treatment conditions which completely inactivate the myrosinase system, while, at the same time, not unacceptably reducing the solubility of the proteins in alkaline media.

Referring to the process according to the invention, the precipitation of proteins in step (c) of the process may be effected in one step or in two steps.

A preferred one-step procedure comprises effecting precipitation of dissolved proteins at a pH of from 3.0 to 6.5 and using, as precipitation aid, an acid polyelectrolyte or a salt of a polyvalent acid.

A preferred two-step procedure comprises

(i) precipitating proteins from the solution by adding an acid in an amount such that the pH of the solution is from 3.5 to 8.5, and separating the precipitate obtained, and

(ii) adding to the solution from which the precipitate has been separated, as a precipitation aid, an acid polyelectrolyte or a salt of a polyvalent acid, and, if necessary, adjusting the pH of the solution to from 3.0 to 6.5 to effect further precipitation of dissolved proteins.

In order that the invention may be more fully understood, reference is made, by way of example, to the accompanying drawings, in which

Figure 1 is a graph on which is plotted, as ordinate, the percentages of nitrogen-containing substances and of phytic phosphorus in defatted rapeseed meal which are extracted at ambient temperature into an aequeous medium, and as abscissa, the pH of the medium,

Figure 2 is a schematic diagram illustrating two preferred manners of carrying out the process of the invention, and

Figure 3 is a graph on which is plotted, as ordinate, the yield of protein isolate, the protein content of the isolate, and the dry matter content of the isolate, obtained by precipitating dissolved proteins from a solution thereof obtained by the extraction procedure employed in the process of the invention, the precipitation being effected by addition of an acid, and, as abscissa, the pH of the medium after addition of the acid thereto.

Thorough investigations have shown that the solubilities of protein and phytic acid vary with pH in a complicated manner, as shown, for example, in Figure 1, in which solubilities are plotted as a function of pH in the case of extraction of defatted rapeseed meal with aqueous solutions at ambient temperature. Investigations have shown that turnip rapeseed and crambe meal have similar solubility profiles. The solubility profiles of seeds of *Brassica* species differ considerably from those of, for example, soybeans. The reason for this is probably that *Brassica* seeds have a very complicated protein composition and contain relatively large amounts of strongly basic proteins of a type not found in soybeans. From the nitrogen solubility profile it can be seen that rapeseed meal contains two large protein fractions with different solubility characteristics. From Figure 1 it can be seen that the maximum solubility of the phytic acid was at around pH 4.8. In the pH range from 4.8 to 8.3, the solubility of phytic acid decreased, probably as a consequence of the formation of poorly soluble complexes with the polyvalent metal ions present in the seed. This is verified by the observation that the sediments obtained after centrifuging the dispersions of the meal in media having a pH in the range from 7 to 11, contained bands of a white precipitate, which was found to consist mainly of metal phytates. At a pH at about 8.3, the solubilities of phytic acid and of nitrogen exhibited minima. In the pH region from 8.3 to about 10, the solubility of both nitrogen and phytic acid increased with increasing pH. Since calcium/magnesium phytates have a low solubility in this pH range, the results imply that phytic acid forms strong complexes with the protein in this pH range. The solubility of phytic acid reached a maximum at a pH close to 10. This maximum occurs in a pH range where the number of positively charged amino acid residues decrease rapidly with an increase in pH. A large proportion of the basic amino acid residues in rapeseed protein are lysine residues, the ε-amino group of which has a pK_a-value of 10.5. The decrease in solubility of the phytic acid between pH 10 and 11 might thus be explained by the dissociation of protein-phytic acid complexes, whereby the phytic acid forms difficultly soluble metal salt complexes. The increase in solubility of phytic acid at pH values greater than 11.5 can be explained by an increased solubility of metal phytates at these pH values. Any phytic acid present in an extract containing the protein will bind to the protein when the protein isolate is precipitated. This means that protein isolates with a high phytic acid content will be obtained from protein extracts with a high content of phytic acid and that protein isolates with a low phytic acid content are

produced from protein extracts with a low phytic acid content.

The marked pH dependence of the phytic acid solubility illustrated in Figure 1 shows that the choice of extraction conditions is of the utmost importance in determining the phytic acid content of the resulting protein isolate. This can be illustrated by the following example. An isolate was prepared by extracting a non-heat-treated rapeseed meal at pH 11.1. After separation of undissolved substances, a protein isolate was precipitated by adding dilute hydrochloric acid and adjusting the pH to 6.6. The precipitated protein isolate contained no detectable amounts of phytic acid. Another protein isolate was prepared by extracting rapeseed meal from the same batch at pH 10.2. After separation of the undissolved substances, a protein isolate was precipitated by adding dilute hydrochloric acid to give a pH of 8.4. The dried product in this case contained 11.5% of phytic acid (calculated as inositol-hexaphosphoric acid).

From Figure 1 it can be seen that the phytic acid solubility has minima at pH values of around 8 and 11. Thus a possible way of producing a protein extract almost free from phytic acid would be to extract rapeseed meal at a pH around 8. Some of the phytic acid is dissolved at this pH, but it has been found possible to suppress the phytic acid solubility, without decreasing the protein solubility, by conducting the protein extraction in the presence of salts of polyvalent metals. Processes based upon a protein extraction at pH about 8, however, suffer from the disadvantage of the relatively low protein solubility at pH 8, and the fact that microbiological growth is much more difficult to avoid at pH 8 than at a pH around 11. For these reasons, the protein solubilisation step in the process of the invention is carried out at a pH in the range of from 10.5 to 12.

The ratio between the amount of defatted seeds and the amount of liquid employed in the extraction step in the process of the invention is not critical. For example, it is possible to work with a meal:liquid ratio of 1:5 or less. To facilitate the protein extraction, it is advisable to grind the defatted seed material further, since shorter extraction times can then be used. The defatted seed material is dispersed in water and an alkali is added until the required pH has been reached. To obtain good protein solubility, it is essential that the meal dispersion is not kept at acid or neutral pH values for too long a time. When the desired pH has been reached, it should be kept constant in the range of from 10.5 to 12, where the solubility of the phytic acid is low. Alternatively, the defatted seed material may be dispersed directly into a basic aqueous solution, and the pH may then be adjusted by further addition of alkali. A draw-back with the latter procedure is that the seed particles have a tendency, under these conditions, to agglomerate and form lumps, surrounded by a gel layer, which obstructs the protein solubilisation.

To raise the pH, any suitable alkaline material may be used, such as sodium hydroxide, ammonia or sodium-orthophosphate. When the seed material contains an insufficient amount of di- and trivalent metal ions to ensure a low phytic acid content in the protein extract, it is preferred to add a sufficient amount of calcium or magnesium salt. By addition of sulphite or ascorbate during the protein extraction, it is possible to obtain an isolate with a somewhat lighter colour than those obtained in the absence of such reducing compounds. The temperature during the extraction stage should be kept as low as possible to avoid protein hydrolysis and other side reactions which can reduce the nutritional value of the product. If the seeds have been exposed to a severe heat treatment, however, it may be desirable to work at an elevated temperature in order to obtain a higher yield. Extraction times of up to 24 hours can be used at ambient temperature without lowering the nutritional value of the protein. The temperature employed during the extraction is important, since when the protein extraction is carried out at higher temperatures, a larger amount of alkali is required to obtain the necessary pH value of 10.5 to 12. For example, if the extraction temperature is raised from 20 to 60°C, the consumption of alkali is increased by a factor of about 10. This also influences conditions employed in the precipitation step, because the amount of acid required to reach the pH at which precipitation occurs increases, and, as a result, the ionic strength of the mother lye will increase, and the amount of precipitation aid required also increases.

When the protein solubilisation is completed, the undissolved material has to be separated. This is preferably performed by centrifuation, but filtration also can be used. It has been found that part of the phytic acid in the alkaline dispersion is present in the form of a very fine precipitate. To obtain a product free from phytic acid, it is essential, therefore, to carry out an effective centrifugation or filtration step. If a multi-step centrifugation or a separate filtration step is applied, complete separation of the phytic acid can readily be obtained.

It is also possible to facilitate separation of the phytic acid by seeding the dispersion. The residue obtained in the separation procedure contains protein which can be recovered in one or more washing steps, which preferably comprise dispersing the solid residue in an aqueous solution of the same pH as used during the extraction, and then separating undissolved materials. Alternatively, the protein extraction can be performed using a counter current extraction procedure.

After separation of the undissolved residue, the proteins may be precipitated from the protein extract in one or two steps. Preferred manners of carrying out such one or two step procedures are illustrated in Figure 2.

In the two-step precipitation procedure (denoted alternative 1 in Figure 2), part of the dissolved protein is precipitated in the first step by addition of an acid. The choice of acid is not critical, but certain acids can give special effects. Thus, precipitation with sulphurous acid or with gaseous sulphur dioxide will give a product with a somewhat lighter colour than precipitation with non-reducing acids. Figure 3 illustrates the variation of the isolate yield (calculated as the ratio between the amount of nitrogen in the isolate and the amount of nitrogen in the extract solution); the protein content of the isolate (N×6.25); and the dry matter content of the precipitate, with the precipitation pH in the first precipitation step. The results plotted in Figure 3 were obtained from experiments with meal from defatted, heat-treated rapeseed extracted at pH 11. From Figure 3 it can be seen that the amount of precipitated protein is relatively independent of the precipitation pH used. The region of technical interest is the pH-range from 3.5 to 8.5. However, it is generally advisable in the first step to use a precipitation pH higher than the precipitation pH in the second precipitation step. The rate of acid addition influences the size and shape of the flocks. If the acid is added slowly with thorough agitation, the precipitate is obtained in a form easy to separate. The precipitated protein isolate is separated by centrifugation or filtration, and the isolate is then washed, preferably several times. To prevent protein loss during the washing steps, the pH of the washing water should be the same as, or slighly lower than, the precipitating pH.

Those proteins which are not precipitated in the first precipitation step are recovered in a second precipitation step by adding a precipitation aid and a pH-adjusting agent so that a pH value between 3.0 and 6.5 is reached. Preferred precipitation aids are:

a. acid polyelectrolytes, such as sodium alginate, peptic acid, carrageenan, furcellaran, carboxy methyl cellulose, polyphosphates, starch, derivatives of starch, or other acid polyelectrolytes suitable for use in food products,
b. salts of polyvalent acids, such as salts of citric acid or cyclic phosphoric acids.

The amount of precipitation aid and the precipitation pH to be used to obtain a maximum yield of the protein isolate in this step, depend on the nature of the precipitation aid and the composition of the protein solution (especially its protein content, ionic strength and ionic composition). The amount of precipitation aid and the precipitation pH are not independent of each other. Thus, at different precipitation pH:s, the maximum isolate yield is obtained when different amounts of precipitation aid are added. The fact that the isolate yield passes through a maximum, for a given precipitation pH, when increasing amounts of precipitation aid are added, is due to the formation of water soluble complexes between the proteins and the precipitation aid when the latter is added in excess. The relation between the isolate yield, the amount of precipitation aid and the precipitation-pH is consistent with the explanation that electrostatic forces play a dominant role in the precipitation of the proteins: a variation in pH influencing the electrical charge of both the precipitation aid and the proteins. The combination of pH and amount of precipitation aid which should be used in each specific case, can easily be determined by experiment. In one case, we found that maximum isolate yield was obtained at pH 5.1, when 0.098 grams of carboxymethyl cellulose were added per gram of dissolved protein, and at pH 4.1, when double the amount of precipitation aid was used.

The amount of precipitation aid bound to the protein isolate varies with the precipitation-pH and the number of acid groups per gram of precipitation aid. The higher the precipitation-pH used, and the higher the average number of acid groups of the precipitation aid per unit weight, the smaller is the amount of precipitation aid bound to the protein in the isolate. The molecular structure (for example, the degree of branching) of the precipitation aid and its molecular weight also influence the amount of precipitation aid which will be bound to the protein isolate.

We have found that the easiest way to obtain a high yield of protein isolate, which is readily separable, and has a homogeneous composition, is to add the precipitation aid in the form of a solution

while agitating. The protein solution should preferably have a pH outside the range in which the precipitation aid is active as a precipitant. After adding the precipitation aid, the pH adjusting compound should be added, while agitating, in a quantity large enough to enable the predetermined pH value of the dispersion to be attained. The structure of the precipitate depends on the rate of which the pH adjusting compound is added. The best result is obtained if it is added slowly and with thorough agitation. The precipitate obtained is separated by centrifugation or filtration and is washed in one or several steps. The washed precipitate constitutes the protein isolate.

Neither the form in which the precipitation aid is added nor the order in which the pH adjusting compound and the precipitation aid are added *per se* determines the isolate yield. Thus, it is also possible to add the precipitation aid as a solid to add the pH adjusting compound before adding the precipitation aid. Various acidic compounds may be used to effect the pH-adjustment, for example organic and inorganic acids, or acidic gases can be used. If reducing acid compounds, such as ascorbic acid, sulphurous acid, or sulphur dioxide are used, the isolate produced will have a somewhat lighter colour than when a non-reducing acid is used.

When a protein isolate is precipitated in one step (alternative 2 in Figure 2), the precipitation aid is added directly to the alkaline protein solution obtained by extraction of the seed material. Then the protein isolate may be precipitated by addition of acid in the same way as described above for the second step of the two-step precipitation procedure. It is, of course, also possible to add the precipitation aid after part of the acid required has been added.

The properties of a protein isolate prepared by precipitation in the presence of a precipitation aid depend on the content of precipitation aid in the isolate, and on the nature of the precipitation aid. Protein isolates, prepared with precipitation aids according to the process of the invention, generally have very high dry matter contents at the pH of precipitation. This is desirable because it simplifies the separation of the newly precipitated isolate and reduces the risk of product losses during subsequent washings of the isolate. By using a suitable washing procedure, an isolate with a neutral taste may be obtained by the process of the invention. We have also found that it is possible to influence the properties of the product by a suitable after-treatment. Thus, by increasing the pH of the isolate after the final washing step, it is possible to impart excellent water-binding, emulsion and foam stabilizing properties to the isolate. When protein isolates are prepared by the two step precipitation procedure, the isolates obtained from each of the two precipitation steps have somewhat different properties. Such isolates can be used separately or as a mixture. The isolates can also be treated by a "washing" with ethanol, acetone or other organic solvents, which can considerably improve the colour and the flavour of the isolates.

In order that the invention may be more fully understood, the following Examples are given by way of illustration only.

Example 1

Rapeseeds containing 8% water were subjected to a heat treatment at 90°C for 18 minutes in an externally heated rotating drum. The myrosinase system (the glucosinolate hydrolyzing enzyme system) was completely inactivated by this treatment. The seeds were crushed in a flaking mill and fat was extracted from the crushed seeds in a conventional extraction apparatus. The defatted product was then ground in a hammer mill. One kilogram of the meal prepared in this way was dispersed in 16.5 litres of deionized water at room temperature. A 0.2 M sodium hydroxide solution was added with agitation until the pH of the dispersion was 11.1. The dispersion was agitated for 30 minutes, while keeping the pH constant by further additions of sodium hydroxide solution. A total of 3.5 litres of sodium hydroxide solution was added. The undissolved material was separated by centrifugation in a batch centrifuge for five minutes at 3100 g. To clear centrifuged protein solution, a 2% solution of sodium hexametaphosphate (obtained from Bdh and consisting mainly of $(NaPO_3)_6$ in water, was added while agitating. 60 ml of the sodium hexametaphosphate solution was added per litre of protein extract. The protein was then precipitated by decreasing the pH of the mixture to 4.9 by the addition of dilute hydrochloric acid while agitating. The precipitate was separated by centrifuging the mixture for five minutes in a batch centrifuge at 3100 g. The precipitate had a dry matter content of 25% and contained 14.5% nitrogen (determined on the dry matter), which corresponds to a protein content of 91% (N×6.25). The isolate was washed by dispersing it in deionized water, and the precipitate was then separated and freeze dried. The dried product has a light brown colour. Using the procedure described above, about 85% of the nitrogen extracted at pH 11.1 was precipitated. Absorption measurements at 280 nm on the supernatant from the precipitation step,

showed that practically all of the proteins extracted at pH 11.1 had been completely precipitated. In total, 0.28 kg dried protein isolate was obtained. The isolate contained less than 0.4 mg phytic phosphorus per gram and less than 0.03 mg glucosinolates or degradation products thereof per gram.

Example 2

A rapeseed protein solution was prepared according to the procedure described in Example 1. To the protein extract was added an aqueous solution containing 1% of carboxymethyl cellulose (Cellugel 3000 Special, SCA, Sweden). 160 ml of the Cellugel solution was added per litre of protein extract. The protein was then precipitated by decreasing the pH of the mixture to 5.1 by addition of dilute phosphoric acid. The mixture was centrifuged for five minutes in a batch centrifuge at 3100 g. The precipitate had a dry matter content of 32%. The isolate was washed in deionized water and freeze dried. The freeze dried isolate had a light brown colour and a nitrogen content of 13.9%, corresponding to a protein content of 89% (N×6.25). About 85% of the nitrogen compounds in the extract was precipitated. A total of 0.27 kg isolate per kilo defatted rapeseed meal was obtained, containing less than 1.0 mg phytic phosphorus per gram and less than 0.06 mg glucosinolates or degradation products thereof per gram.

Example 3

A rapeseed protein extract was prepared according to the procedure described in Example 1. To the protein extract was added a 1% solution of Cellugel 3000 Special in water while agitating. 320 ml Cellugel solution was added per litre of protein extract and the protein was then precipitated by reducing the pH of the mixture to 4.1 using dilute phosphoric acid. The mixture was centrifuged for five minutes in a batch centrifuge at 3100 g. The dry matter content of the isolate was 29.5%. The isolate was washed in deionized water and freeze dried. The dried product had a light brown colour. The nitrogen content of the isolate was 13.1%, which corresponds to a protein content of 82% (N×6.25). About 90% of the nitrogen-containing compounds of the protein extract were precipitated by the procedure described. A total of 0.30 kg isolate per kilogram defatted rapeseed meal was obtained. The isolate contained less than 1.3 mg phytic phosphorus per gram and less than 0.08 mg glucosinolates or degradation products thereof per gram.

Example 4

A rapeseed protein extract was prepared according to the procedure described in Example 1. To the protein extract was added a 0.5% solution of λ-carrageenan in water while agitating. 320 ml of the λ-carrageenan solution was added per litre of protein extract, and the protein was then precipitated by reducing the pH of the mixture to 5.3 with hydrochloric acid. The mixture was then centrifuged for five minutes in a batch centrifuge at 3100 g. The dry matter content of the isolate was 13%. The isolate was washed and freeze dried. It had a light brown colour and its nitrogen content was 13.3% of the dry matter, corresponding to a protein content of 83% (N×6.25). A total of 0.34 kg isolate per kilogram defatted meal was obtained. The isolate contained less than 0.6 mg phytic phosphorus per gram and less than 0.08 mg glucosinolates or degradation products thereof per gram.

Example 5

A rapeseed protein extract was prepared according to the procedure described in Example 1. To the protein extract was added 0.1 M hydrochloric acid until the pH of the mixture was 6.6. The precipitate formed was removed by centrifugation in a batch centrifuge at 3100 g for five minutes. The supernatant was a transparent yellow solution. The precipitate, which had a dry matter content of 23%, was washed with deionized water to which was added 0.1 M hydrochloric acid until the pH was just under 6.6. The isolate was freeze dried. It then had a brown colour and contained 14.0% nitrogen, which corresponds to a protein content of 87.5% (N×6.25). About 40% of the nitrogen compounds dissolved at pH 11.1 was precipitated in this way. A total of 0.14 kg dried protein isolate was obtained. Phytic acid could not be detected in the product. The isolate contained less than 0.03 mg glucosinolates or degradation products thereof per gram.

To the yellow solution obtained as described above by separation of the precipitate was added a 1% solution of Cellugel 3000 Special in deionized water. 120 ml Cellugel solution was added per litre of protein extract. The solution was agitated and hydrochloric acid was added until the pH of the mixture was 5.0. The precipitate formed was recovered by centrifugation for five minutes in a batch centrifuge at 3100 g. By this procedure about 80% of the nitrogen compounds in the yellow protein solution were precipitated. The precipitate, which had a dry matter content of 35%, was washed with dionized water and freeze dried. It had a yellow colour and its nitrogen content was 15.0%, which corresponds to a protein content of 94% (N×6.25). 0.16 kg of the dry product was obtained by this precipitation

step. Adsorption measurements at 280 nm showed that the supernatant from this precipitation step had a very low protein content, and that an almost complete precipitation of the proteins dissolved at pH 11.1 had been achieved. The isolate contained less than 0.9 mg phytic phosphorus per gram and less than 0.03 mg glucosinolates or degradation products thereof per gram.

Example 6

70 kg defatted, heat-treated-rapeseed meal was dispersed in 680 litres deionized water at room temperature. The rapeseed meal used had a dry matter content of 94% and its nitrogen content was 6.5% of the dry matter. The meal had a PDI-value of 33% and the nitrogen solubility at pH 11.5 was 69% (PDI stands for Protein Dispersibility Index, determined according to A.O.C.S. Tentative Method Ba 10—65, corrected 1967). To the dispersion was added, while agitating, a 2 M sodium hydroxide solution until the pH of the dispersion was 11.2. The dispersion was agitated for 45 minutes, while keeping its pH constant by adding sodium hydroxide solution. A total of 20 litres of 2 M NaOH was added. The undissolved material was removed in a continuous centrifuge. A total of 230 kg sludge with a dry matter content of 16.5% was obtained. 27 litres of a 2% solution of sodium hexametaphosphate in water (BDH quality) was added to 230 litres of the supernatant while agitating. The protein was precipitated by addition of 5 litres of 1.8 M HCl, whereupon the pH of the mixture was 4.9. The precipitate was separated in a continuous centrifuge. By analyzing the streams to and from the continuous centrifuge it was found that 77% of the nitrogen content of the stream going to the centrifuge was separated in the form of a precipitate. The precipitate was washed twice with deionized water. The isolate losses during the washing steps were very small and the washing water was practically free from suspended material. The dried precipitate had a beige colour and contained 14.5% nitrogen, which corresponds to a protein content of 90.6% (N×6.25). The isolate contained less than 0.4 mg phytic phosphorus per gram.

Example 7

To 230 litres of the alkaline protein solution obtained as described in Example 6 was added, while agitating, 72 litres of a 1% aqueous solution of carboxy methyl cellulose (Cellugel 3000 Special, SCA, Sweden). The protein was precipitated by decreasing the pH of the mixture to 5.1 by addition of hydrochloric acid while agitating. A total of 10 litres of 1.8 M HCl was added. The precipitate was separated in a continuous centrifuge. Analysis of the streams to and from the centrifuge showed that 82% of the nitrogen content in the input stream to the centrifuge was recovered in the form of a precipitate. The precipitate was washed with deionized water and was recentrifuged. The washing water from the centrifuge was free from suspended material. The precipitate was spray-dried and had a beige colour. The nitrogen content of the precipitate was 14.1%, which corresponds to a protein content of 88% (N×6.25), and the precipitate contained less than 1 mg phytic phosphorus per gram.

Example 8

To 515 litres alkaline protein extract, prepared according to the procedure described in Example 6, dilute hydrochloric acid was added, while agitating, until the pH of the mixture was 6.6. A total of 30 litres of 0.5 M HCl was added. The precipitate formed was separated in a continuous centrifuge. Analysis of the streams to and from the centrifuge showed that 35% of the nitrogen content in the input stream to the centrifuge was separated in the form of a precipitate. The precipitate was washed in deionized water and was spray-dried. It had a beige colour and a nitrogen content of 15.2%, corresponding to a protein content of 95.0% (N×6.25). The phytic phosphorus content of the isolate was less than 0.2 mg per gram.

To 470 litres of the supernatant obtained after separating off the protein precipitate, 130 litres of a 1% solution of Cellugel 3000 Special in deionized water was added while agitating. Dilute hydrochloric acid was added, while agitating, until the pH of the mixture was 4.9. A total of 15 litres of 0.5 M HCl was added. The precipitate formed was separated by centrifuging in a continuous centrifuge and was washed in deionized water. The precipitate was very easy to separate and the isolate losses during the washing were very small. The precipitate was spray-dried. It had a beige colour and its nitrogen content was 14.3%, which corresponds to a protein content of 89.4% (N×6.25). The phytic phosphorus content of the product was less than 0.9 mg per gram.

Example 9

One kilogram of heat-treated defatted rapeseed meal was dispersed in 13 litres deionized water at a temperature of 40°C. 0.2 M sodium hydroxide solution was added while agitating, until the pH of the dispersion was 11.0. The dispersion was agitated for 30 minutes at 40°C, the pH

being maintained at 11.0 by further addition of sodium hydroxide solution. A total of 7 litres of sodium hydroxide solution was added. The undissolved meal residue was separated by centrifuging in a batch centrifuge for five minutes at 3100 g. To the supernatant, a 1% solution of carboxymethyl cellulose in water (Cellugel 3000 Special, SCA, Sweden) was added while agitating. 160 ml of the CMC-solution was added per litre of protein extract. The protein was precipitated by decreasing the pH of the mixture to 4.6 by adding dilute hydrochloric acid while agitating. The precipitate was separated by centrifuging for five minutes in a batch centrifuge at 3100 g. The precipitate had a dry matter content of 23.2% and a nitrogen content of 13.7% calculated on dry matter, which corresponds to a protein content of 85.7% (N×6.25). The isolate was washed by dispersion in totally deionized water, and the precipitate separated and freeze dried. The dried product had a light brown colour. By the procedure described above, about 87% of the nitrogen compounds dissolved at pH 11.0 were precipitated. A total of 0.30 kg isolate per kilogram defatted rapeseed meal was obtained. The phytic phosphorus content of the isolate was less than 1 mg per gram.

WHAT WE CLAIM IS:—

1. A process for the preparation of a protein isolate substantially free from phytic acid (as herein defined), which comprises:

(a) extracting protein from defatted seeds of the genera *Brassica* or *Crambe* in an aqueous medium having a pH in the range of from 10.5 to 12 and a temperature of from 0 to 70°C,

(b) separating non-dissolved material to obtain a solution containing dissolved proteins,

(c) precipitating proteins from the solution by adding an acid and a precipitation aid,

(d) separating the precipitate obtained, and

(e) washing the precipitate to remove remaining mother liquor.

2. A process according to claim 1, in which the seeds are subjected to a heat-treatment to deactivate myrosinoses therein prior to step (a).

3. A process according to claim 1 or 2, in which one or more salts of a polyvalent metal are present in the aqueous medium into which proteins are extracted in step (a), the amount of such salts being such as to provide sufficient polyvalent metal ions to convert phytic acid (as herein defined) present in the defatted material into an insoluble form.

4. A process according to any of claims 1 to 3, in which the polyvalent metal is calcium and/or magnesium.

5. A process according to any of claims 1 to 4, in which step (c) comprises:

(i) precipitating proteins from the solution by adding an acid in an amount such that the pH of the solution is from 3.5 to 8.5, and separating the precipitate obtained, and

(ii) adding to the solution from which the precipitate has been separated, as a precipitation aid, an acid polyelectrolyte or a salt of a polyvalent acid, and, if necessary, adjusting the pH of the solution to from 3.0 to 6.5 to effect further precipitation of dissolved proteins.

6. A process according to any of claims 1 to 4, in which precipitation of dissolved proteins in step (c) is effected in one step at a pH of from 3.0 to 6.5, and in which the precipitation aid is an acid polyelectrolyte or a salt of a polyvalent acid.

7. A process according to claim 5 or 6, in which the acid polyelectrolyte is carboxymethylcellulose, a polyphosphate, a hexametaphosphate, an alginate, pectic acid, carrageenan, furcellaran, starch, or a starch derivative.

8. A process according to claim 5 or 6, in which the salt of a polyvalent acid is a salt of citric acid or a salt of a cyclic polyphosphoric acid.

9. A process according to any of claims 1 to 8, in which the non-dissolved material separated in step (b) is redispersed in an aqueous alkaline solution having a pH in the range of from 10.5 to 12 in order to extract soluble protein remaining in the non-dissolved material.

10. A process for the preparation of a protein isolate, substantially as herein described in any of the Examples.

11. A protein isolate obtained by the process claimed in any of claims 1 to 10.

12. A food product formed from a protein isolate as claimed in claim 11.

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3 SHEETS *This drawing is a reproduction of the Original on a reduced scale*

Sheet 1

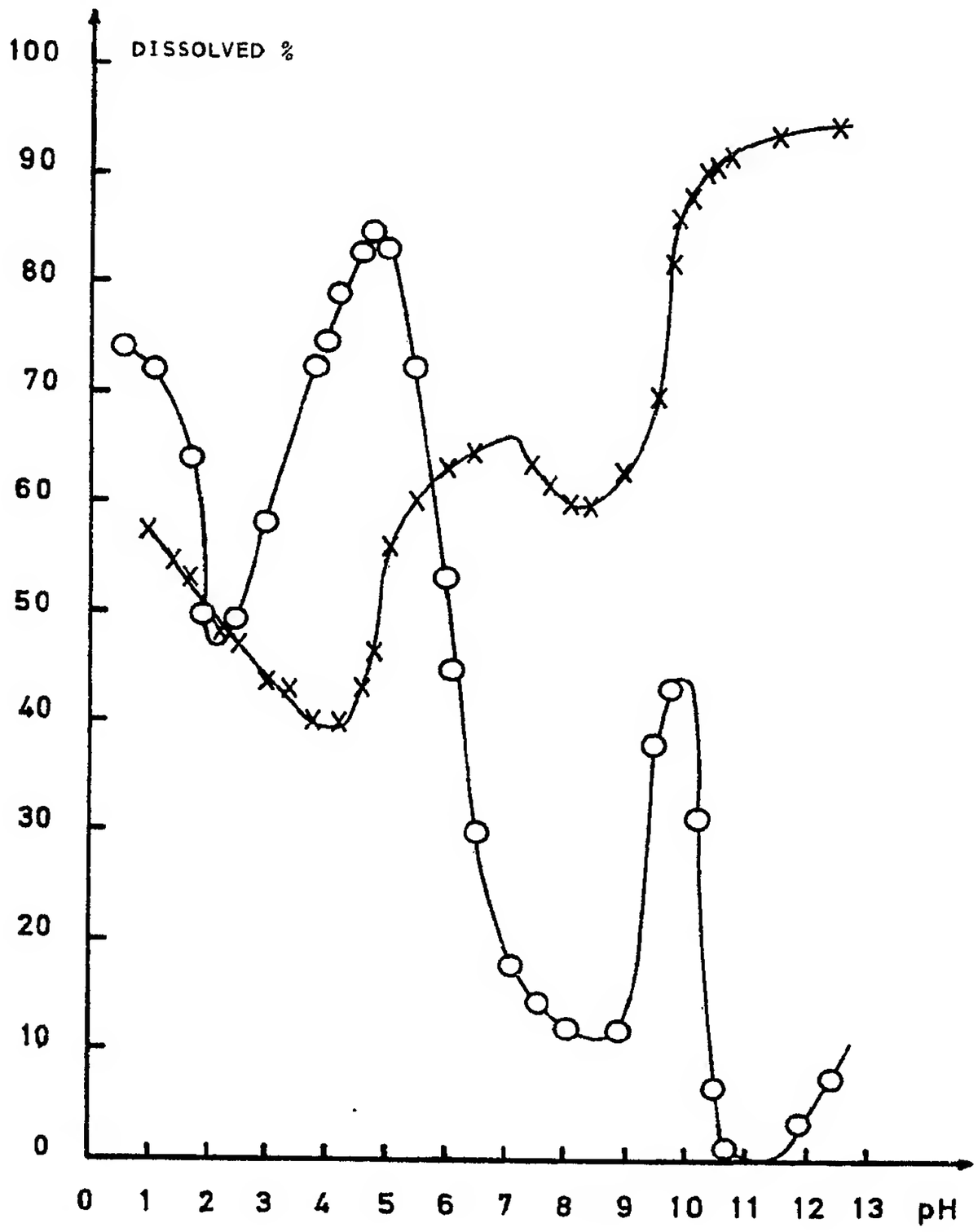

Fig.1 SOLUBILITY OF NITROGEN CONTAINING SUBSTANCES (X) AND PHYTIC PHOSPHORUS (O) AS A FUNCTION OF PH.

1480857 COMPLETE SPECIFICATION

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Sheet 3

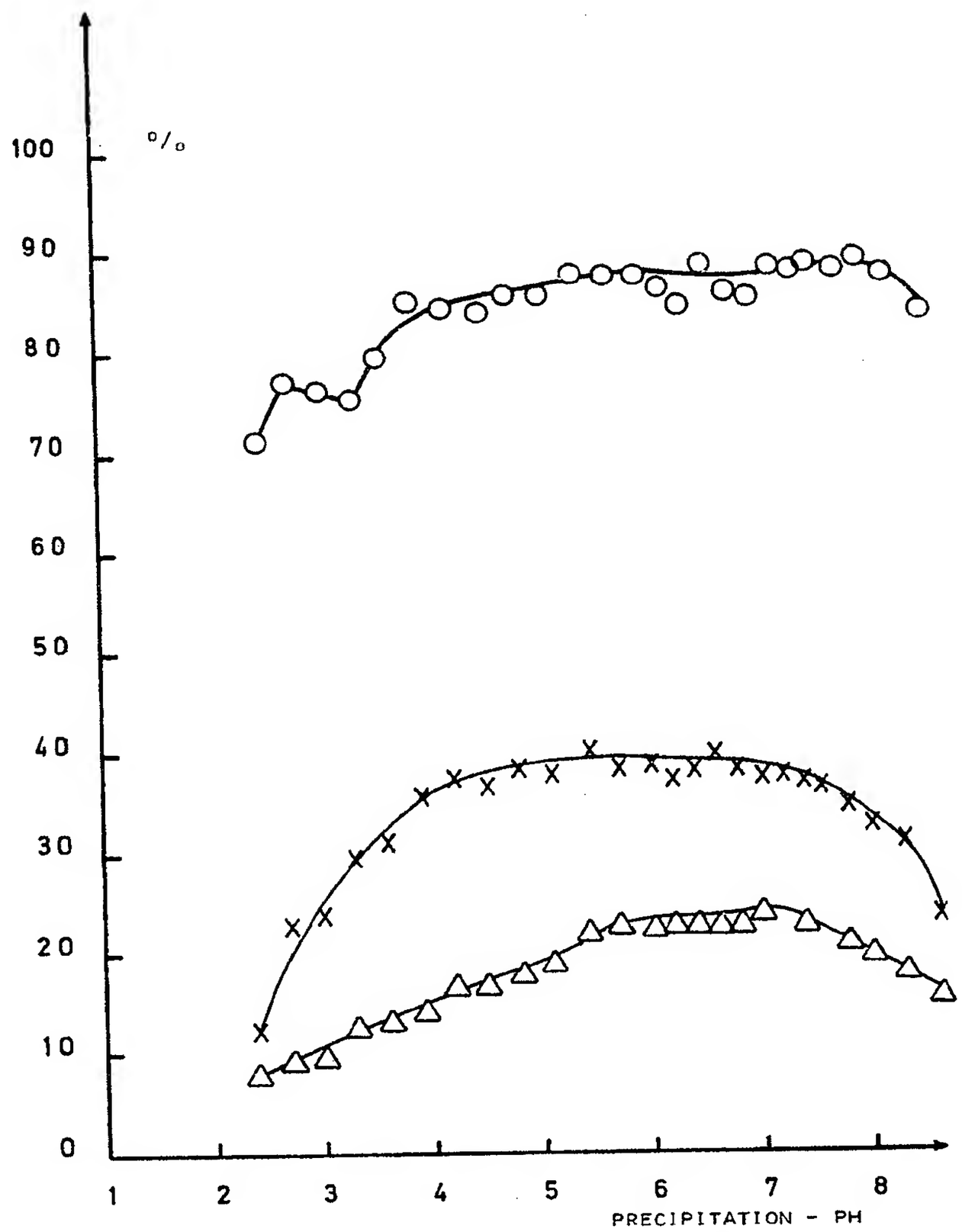

Fig.3 YIELD OF ISOLATE (X), PROTEIN CONTENT (O) AND THE DRY MATTER CONTENT OF THE ISOLATE (Δ) AS A FUNCTION OF PH IN THE FIRST PRECIPITATION STEP.

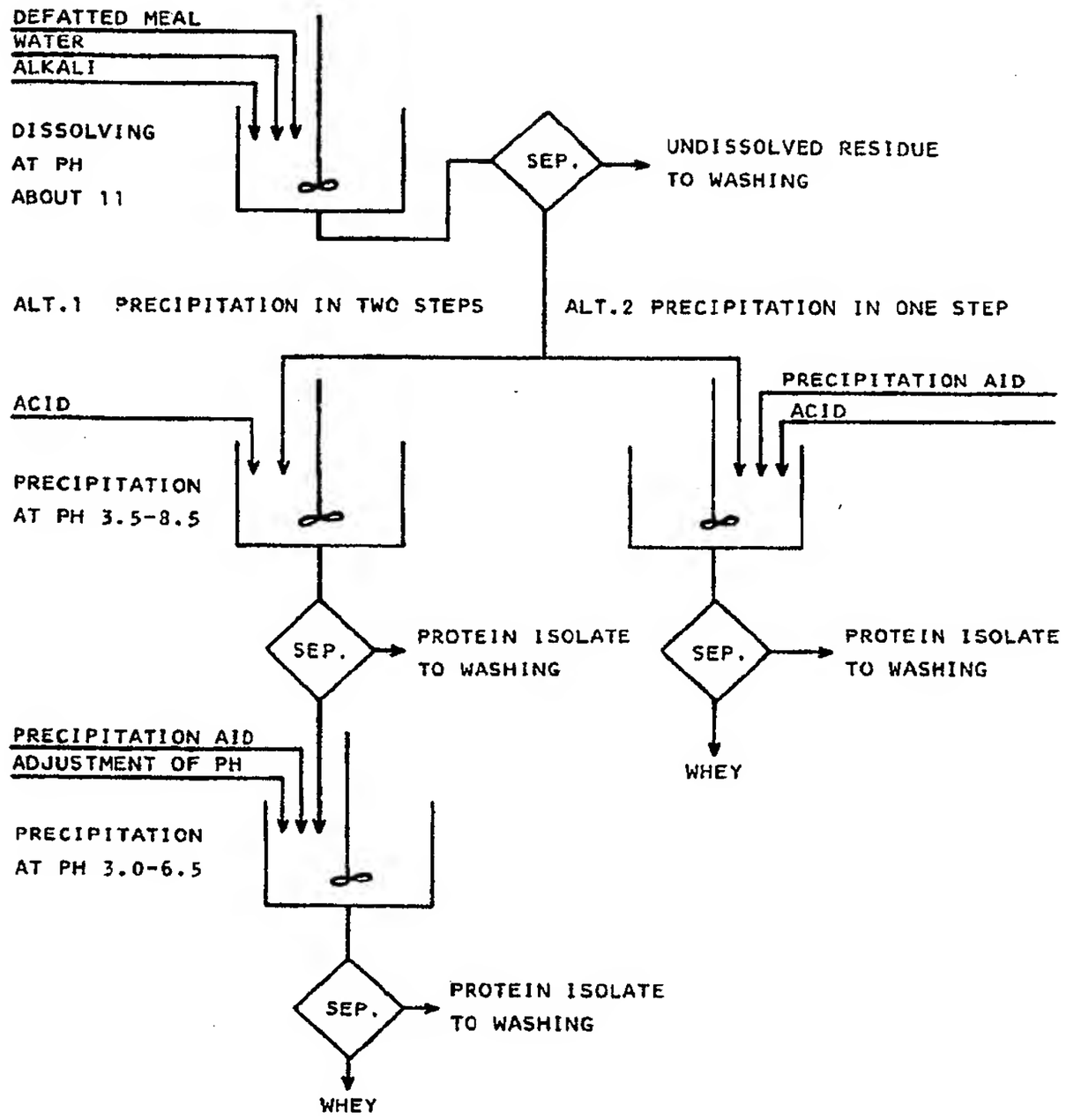

Fig.2